

University of Chicago

THE PROBLEM OF CASTES AND
CASTE DIFFERENTIATION
IN PRORHINOTERMES
SIMPLEX (HAGEN)

A PART OF A DISSERTATION SUBMITTED
TO THE FACULTY OF THE DIVISION OF
THE BIOLOGICAL SCIENCES IN CANDIDACY
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

1941

By

ELWOOD MORTON MILLER

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THE PROBLEM OF CASTES AND CASTE DIFFERENTIATION IN PRORHINOTERMES SIMPLEX (HAGEN)

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TWO VIEWS have existed during discussions of the problem of caste differentiation in termites. Thompson (1917, 1919, 1922), Thompson and Snyder (1920), Imms (1919), and Bathellier (1927) have held that differentiation is a genetically-directed event, unalterable by extrinsic factors, and that young nymphs could be assigned by morphological criteria to the various caste types at hatching. On the other hand, Grassi and Sandias (1896-97), Jucci (1924), Heath (1927, 1931), and Kalshoven (1930) believed the nymphal or immature forms to be susceptible to environmental influences which might determine the direction of differentiation. Hare (1934), likewise, was unable to find evidence that sterile (soldier) and reproductive types were differentiated at hatching in the species *Reticulitermes arenincola*. Pickens' (1932, 1938) and Castle's (1934) observations gave support to the "extrinsic theory" and in addition suggested a mechanism which might be involved in differentiation and in the regulation of caste proportions. Castle extracted a substance from functional reproductives of *Zootermopsis* which, when fed to nymphs, had a sex and caste specific inhibiting effect on further differentiation of the nymphs towards a functional reproductive condition. It was shown also that a special salivary diet received from other members of the colony could not be the cause of reproductive differentiation. Furthermore, the introduction of soldiers into an incipient colony delayed the production of other soldiers.

It seemed desirable to pursue further this question of caste formation,

*The writer wishes to acknowledge his indebtedness and express his gratitude to Professor A. E. Emerson and to Professor W. C. Allee for extensive encouragement and valuable criticism during the course of these studies. Thanks are due Professor Sewall Wright for statistical advice. Appreciation is also given the University of Miami for certain opportunities and facilities during the course of the work.

using a species of termite in which the colony structure and organization had not been frequently examined. It appeared possible that various degrees of plasticity of nymphs may exist in the different families of termites, or that differentiation becomes expressed at different nymphal ages or under different stimuli in various genera.

MATERIAL AND METHODS

Prorhinotermes simplex (Hagen) is a rather primitive member of the family Rhinotermitidæ. The genus is found the world around but only within the tropics, and to date is reported only from near sea coasts or on islands. The species used is the only species of the genus which has been reported from the United States and is apparently confined to a narrow zone along the southeast coast of Florida. No colonies have been found further inland than five miles. Colonies in nature are characteristically "damp wood" inhabitants, i.e., they require considerable moisture but do not utilize subterranean habitats. Soldiers are comparatively abundant within each colony, and third-form reproductives are common. No functional second-form reproductives have been found in nature in Florida. Winged reproductives were first collected in the state by the present investigator. This species of termite appears to be unique in the form of wing pads possessed by certain members of the colony. These were described by Thompson and Snyder (1920) but the potentialities of this type of nymph were heretofore not investigated. Thompson had assumed that these were nymphs of second-form reproductives, but had no developmental evidence therefor.

Material for this study was collected in Dade County, Florida.* Colonies or colony portions were stored, until used, in large jars and supplied with original wood and frass from the collecting site.

Any special details of procedure will be noted in conjunction with later descriptions of experiments. In general, the methods of Grassi and Sandias were followed and attempts were made to segregate small groups of recorded constitution and to follow the functional and structural changes, if any, of the individuals within the group. Mortality of these small groups was high, possibly because of nutrition maladjustments (Hendee, 1934) and partly because of mite infestations. Without special study on the problems it was eventually concluded that in culturing

*The writer wishes to express his thanks to Mr. R. F. Dodd of the Murphy Termite Company, Miami, Florida, for his coöperation and aid in supplying part of the living material.

these small groups it is advisable to (1) provide "conditioned" wood or frass from the original colony; (2) to arrange this in the container in such contact with a moisture supply that the wood absorbed moisture (these termites cannot obtain sufficient moisture from the air nor depend wholly on metabolic water); (3) to arrange the contents of the container

TABLE 1

RANGES OF MEASUREMENTS IN PRORHINOTERMES SIMPLEX (HAGEN)
(in millimeters)

COLONIES M-3, 4, 7:

Antennal seg.	10	11-12	13	14	15	16	17	18
Head	0.488-	0.549-	0.762-	1.006-	0.976-	1.098-	1.159-	1.098-
Width	0.549	0.702	0.884	1.098	1.219	1.249	1.280	1.280
Pronotum	0.244-	0.335-	0.427-	0.630-	0.640-	0.701-	0.793-	0.732
Width	0.366	0.549	0.579	0.671	0.854	0.884	0.915	1.280
Mesonotum	0.305-	0.427-	0.488-	0.671-	0.732-	0.823-	0.915-	0.762-
Width	0.427	0.549	0.610	0.800	0.976	1.067	1.098	1.098

COLONIES M-14, 16, 18:

Antennal seg.	10	11-12	13	14	15	16	17	18
Head	0.459-	0.594-	0.756-	0.864-	1.026-	1.053-	1.080-	1.134-
Width	0.513	.0702	0.891	1.026	1.134	1.215	1.269	1.296
Pronotum	0.243-	0.351-	0.405-	0.459-	0.648-	0.702-	0.729-	0.810-
Width	0.324	0.405	0.567	0.648	0.783	0.864	0.945	1.026
Mesonotum	0.297-	0.405-	0.459-	0.513-	0.702-	0.756-	0.918-	0.945-
Width	0.405	0.513	0.594	0.729	0.918	0.999	1.188	1.269
Mean Head	0.486	0.642	0.822	0.958	1.069	1.132	1.196	1.218
Width	$\pm .001$	$\pm .002$	$\pm .002$	$\pm .003$	$\pm .002$	$\pm .0057$	$\pm .004$	$\pm .008$
Stand. Dev.	0.0116	0.0314 ⁺	0.0224 ⁺	0.0421 ⁺	0.0185 ⁺	0.046 ⁺	0.034	0.0325
Stand. Error of Deviation	0.001 ⁺	0.004 ⁺	0.0001	0.002 ⁺	0.001 ⁺	0.004	0.002 ⁺	0.005 ⁺
Head Width Growth Increment.								
	1.313	1.294	1.199	1.079	1.064	1.056	1.018	
Mean Mesoth. Width	0.346	0.437	0.509	0.645	0.798	0.910	1.028	
	$\pm .005$	$\pm .004$	$\pm .004$	$\pm .003$	$\pm .005$	$\pm .007$	$\pm .006$	
Stand. Dev.	.0284 ⁺	.0238 ⁺	.033 ⁺	.040 ⁺	.049 ⁺	.059 ⁺	.054 ⁺	
Stand. Error of Mean	.005 ⁺	.004 ⁺	.004 ⁺	.003 ⁺	.005 ⁺	.007 ⁺	.006 ⁺	
Stand. Error of Deviation	.004 ⁺	.003 ⁺	.002 ⁺	.002 ⁺	.003 ⁺	.005 ⁺	.004 ⁺	

so that moisture films and "trap" corners of smooth substratum do not occur. The importance of the last item was not appreciated early, hence many individuals undergoing ecdysis became isolated on such a spot and, failing to grip the substratum at critical moments in moulting, died, decayed, and caused a further sticky trapping medium. This phenomenon may become accumulatively detrimental.

It was desired in this project to test the potentiality of nymphs and the possibility of an inhibiting influence by supplementary reproductives

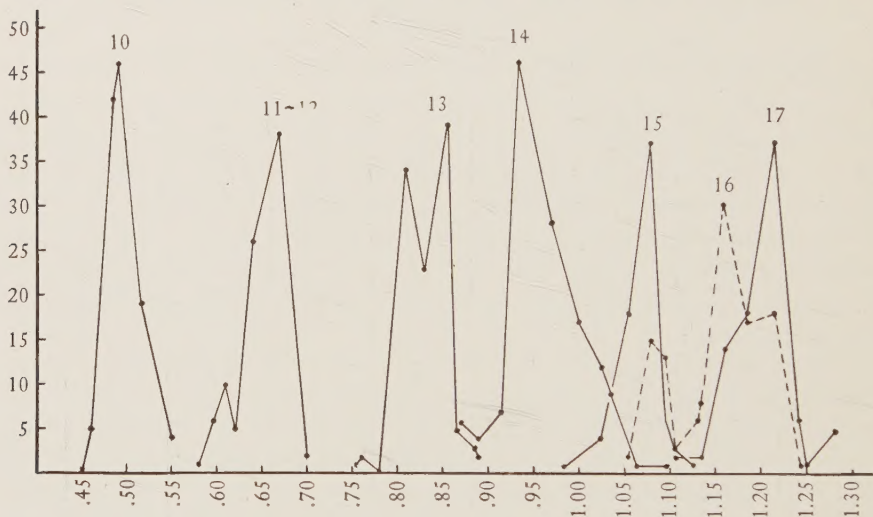


Fig. 1. Frequency distribution with regard to head widths, of measurements of 715 individuals from colonies M-3, 4, 7, 14, 16, 18. Number of antennal segments possessed by each group is indicated by the figures near the apex of each curve. Head widths are in millimeters on the horizontal axis; number of individuals on the vertical axis. (Dotted line is merely for distinction from adjacent lines.)

and soldiers upon small segregated groups. This was not practical, however, until some observations had been attempted to discern whether caste lines could be distinguished in young immature individuals, as in Hare's (1934) work on *Reticulitermes*, and to identify if possible the various instars of *Prorethra simplex* in relation to developmental potentialities.

Consequently, measurements were made by ocular micrometer on seven hundred fifteen individuals and the results were plotted in distribution polygons. These are summarized in Figures 1, 2, and 4, and in Table 1.

OBSERVATIONS

A. SIZE DIFFERENTIATION WITHIN COLONIES

Prorhinotermes simplex hatches from the egg with ten antennal segments and a head-width average of $0.486 \pm .001$ mm. These first instar individuals form a distinct and homogeneous group, and specimens of this stage are easily segregated from others even by gross examination. Care must be taken in such sorting, however, not to be distracted by body length, since this may vary from 0.945 mm. to 1.350 mm. If sorting is

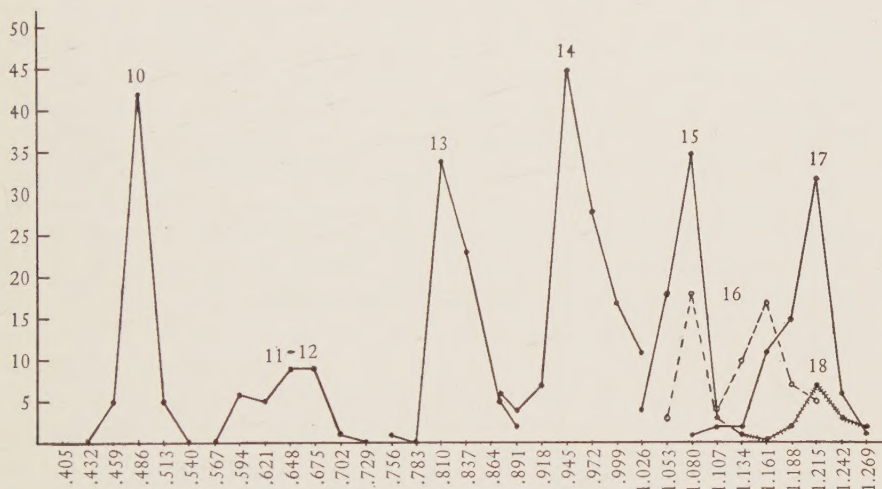


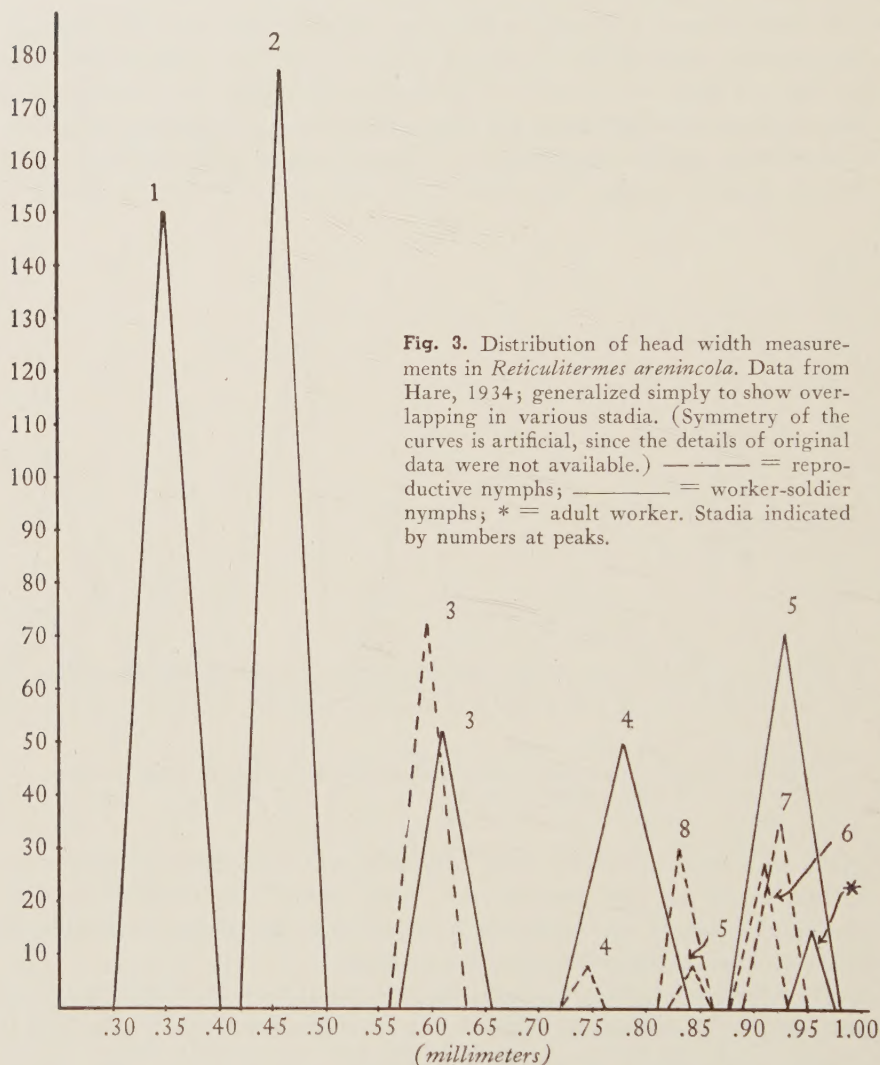
Fig. 2. Frequency distribution of head widths of specimens from colonies M-14, 16, 18 only. Symbols as in Fig. 1.

made on a basis of general body appearance or size, then two instars are included and one has together the "wide-headed" and "narrow-headed" individuals which are sometimes referred to in the literature holding to a thesis of caste differentiation at hatching.

Transition from first to second instar with the concomitant changes in head-width and antennal segment number has been directly observed in several cases, and the implications in the first two distributions in Figure 1 are thus strengthened. The first instar is apparently quite short; some individuals attain the second stage within a week after segregation.

The progression of measurements through successive instars is, as indicated in Figure 1, distinct until individuals having 15 antennal segments

are encountered. Here the rate of head-width increase slows down until considerable overlapping of measurement occurs. The complete signifi-



cance of this is not yet analyzed. It is not unusual among insects to find such a departure from Dyar's rule, as Gaines and Campbell (1935) have shown. A similar retardation and even a regression in head size as older

instars are reached is seen upon examination of Hare's (1934) measurements (Figure 3) for *Reticulitermes* and in Heath's (1927) measurements on *Zootermopsis*. This decrease of head-width may be related in termites to the physiology of caste differentiation and with general nutrition. The possibility of the latter factor is suggested after noting Heath's observations that small young colonies had, on the whole, individuals of smaller head-widths. In the present case of *Prorhinotermes* some of the overlapping may be due to the fact that remnants of several different colonies were used as material for measurement. The slight bimodality in the third instar is due to this mixing, as examination of the original data shows, and it disappears when colonies M-3 and M-4 are omitted. [That colonies may differ noticeably in respect to mean head measurements has been shown by Emerson (1935) for *Nasutitermes guayanae*.] Individuals from the other colonies do not produce this bimodal distribution (Figure 2). This technicality does not explain, however, the position of the group with sixteen antennal segments (Figure 1). A few of these from colony M-14 may belong in the fifteen antennal segment classification, since difficulty was experienced in classifying some individuals due to the poorly delineated character of the third and fourth antennal segments, but even with colony M-18 alone there was great transgression of the head-widths of this group upon both those with fifteen and those with seventeen antennal segments.

The similarity of this situation to that seen in the distribution of worker head-widths in Hare's measurements on *Reticulitermes* suggests that we may have here measurements for a worker caste. Thompson and Snyder (1920) called *Prorhinotermes simplex* individuals having sixteen antennal segments "workers." However, my preliminary experiments in which such individuals were segregated showed that they could develop, within a month, into third-form reproductive types.

Judging, then, from the distribution frequency of these measurements there are six and possibly seven instars in this species preceding the wing-padded condition; one other instar appears to intervene between the wing-pad and alate stages. There is, furthermore, no reliable clue in head-width measurement alone to the beginning of caste differentiation. This confirms Miss Hare's findings on *Reticulitermes arenicola*, with the exception that she found that workers in the fifth stadium had larger heads than reproductive nymphs in the same stage, but the fact that only eight reproductive nymphs of this stage were measured may throw some question on the reliability of this difference. Her primary distinction between reproductive and sterile lines rested on the beginning of wing

buds at the third stadium. Once this distinction had been made, then further analysis showed some differences in average head-widths, but the assorting of unknown individuals from a natural colony could not have been accomplished except in terms of mesonotum development. (Figure 3.)

Similar tabulations and graphing of measurements of pronotum width showed trends much the same as those just discussed for head-widths. Slightly more overlap was detected between first and second and second and third instars, but at least part of this may be due to the error liable in measuring the pronota of these young nymphs; a uniform angle of

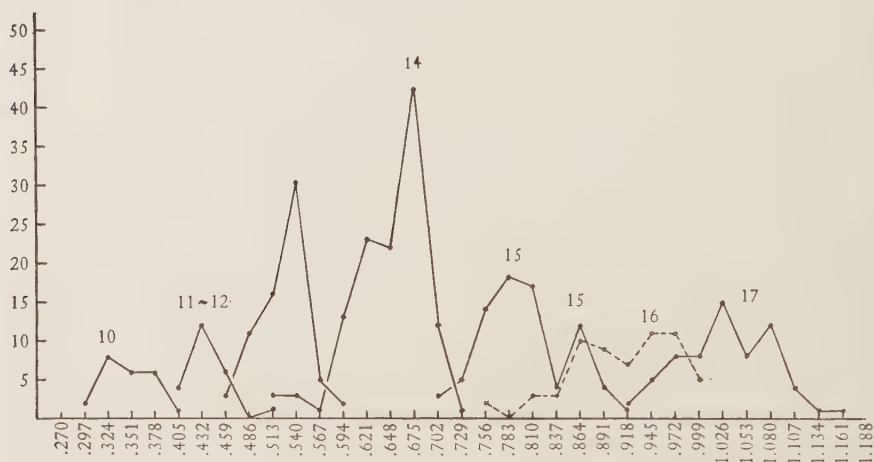


Fig. 4. Frequency distribution of individuals from colonies M-14, 16, 18 in relation to mesonotum widths. Symbols as in Fig. 1.

observation is difficult to maintain. Distinct unimodal curves are indicated for the first four instars, but thereafter the postulated effects of nutrition and caste differentiation obscure the picture. A slight bimodality results at the sixteen antennal segment stage, but only as a result of adding colonies M-14, M-16, and M-18. M-18 alone, in which most of the measurements were made, shows a unimodal distribution at this stadium.

Recalling that in Hare's observations on *Reticulitermes* the only external criterion by which to separate the reproductive and sterile castes after the second stadium was the presence of wing buds, measurements of mesonota from about four hundred *Prorehinotermes* were plotted to show frequency distribution. Results are seen in Figure 4.

Mesonotal widths were recorded instead of the diagonal distances used by Hare. The unique type of wing pad found in *Prorehinotermes simplex*

and the lack of any posteriorly growing wing buds made this seem advisable. These wing pads, figured by Thompson and Snyder (1920), appear to be latero-posterior expansions of the mesonota and metanota, but curve medially so that right and left pads appear to fuse at their posterior borders. If growing wing pads be taken as a sign of a reproductive line of development then the measurements of mesothorax-width should serve in detecting the instar at which the reproductive caste begins differentiation.

But here again the progression of characteristic measurements is rather uniform and unimodal until the stage with fifteen antennal segments is reached. Bimodality appears at this stage and is continued through the remainder of the stadia. Whether this bimodal distribution of mesonota measurements indicates beginning external differentiation between reproductive and sterile castes is somewhat uncertain. The difference between the two modes of the fifteen-antennal-segment group, for example, is very small—being of the order of 0.08 mm.—and the disparity between the modes does not tend to increase in later stadia as might be expected if wing pads were developing in one caste line and not in the other. Definitive wing pads could not be detected in any of the individuals included in the graph in Figure 4. In other words, there is not any observable, definite enlargement of wing pads from stadium to stadium, as is the case in wing bud formation in the fifth to seventh stadia of *Reticulitermes* or in the *Kalotermitidæ*. Individuals having detectable wing pads all appear to fall in the same size class, with great regularity in head-size, antennal segments and mesonotal-width. These individuals then have had a mesonotal-width increase of about 42 per cent over the largest non-wing-padded group in the colony. Between any other successive groups the mesonotal increase is not over 19 per cent. Thus the wing pads appear to develop rather suddenly. This situation seems to be unique to this genus.

As a result of these exploratory measurements one is left with the conclusion that if reproductive and sterile lines of development are differentiated early in the *Prorhinotermes simplex* life history there is little or no external morphological sign of it in either head-width, pronotal, or mesonotal-growth-rates. At least, the differences are sufficiently minute as to be impractical for regular detection and use upon living animals.

With the caste differentiation picture thus in an unsatisfactory state, it was decided to test potentialities of each growth group by isolating it and following subsequent developmental and growth events. In view of the lack of early morphological indications of caste differences it was thought possible that in this species differentiation may set in much later

than in *Reticulitermes*. The comparative scarcity of first and second stadia and the difficulty of raising them in small groups forbade a hunt for caste differentiation beginnings from the start of development, and these considerations led to an attempt to trace backwards in the growth-stage series to whatever threshold for differentiation might exist. Because of the distinctness of the group and the ease of distinguishing them from other nymphs, the wing-padded forms were selected first for these tests.

B. EXPERIMENTS ON POTENTIALITIES OF WING-PADDED NYMPHS

The general anatomy of this form has been described by Thompson and Snyder (1920) under the heading of "second-form nymph." As will be shown later this seems to be an improper designation for this type of individual, but the general anatomical account appears correct. These individuals have seventeen to eighteen antennal segments; the wing pads appear as glossy shields on the meta- and mesothorax and already have

TABLE 2
SUMMARY OF MEASUREMENTS ON WING-PADDED FORMS
(in millimeters)

ANTENNAL SEGMENTS:	17	*17*	18
Head-width range	1.242—1.296	1.215—1.269	1.242—1.323
Pronotum-width	0.999—1.026	0.945—1.026	0.945—1.053
Mesonotum-width	1.458—1.485	1.404—1.512	1.404—1.512
Metanotum-width	1.431—1.458	1.377—1.431	1.404—1.431

Mean head-width = 1.255 mm.

Mean mesonotum-width = 1.469 mm.

17 indicates an immature, unbristled segment between segments III and IV.

indicated within them the primary venations of winged adults. Typical measurements are summarized in Table 2. These forms may or may not contain abundant protozoa, but those which do may be readily detected by the darkened intestine which shows through the dorsal wall of the abdomen. Small groups of these nymphs live well under laboratory conditions and are active in building galleries and in sealing off living space in corners of the containers.

In October and November some colonies contain individuals having swollen but not elongated wing pads, and pigmented eyes. When put into an observation colony of worker nymphs and soldiers this type of wing-

padded form may soon develop into alates. Such individuals have been encountered only during the months mentioned and this particular stadium is apparently of short duration. Similar forms are found in a sample of *P. simplex* from Puerto Rico.

Homogeneous groups of such wing-padded individuals were placed in three-quarter ounce jars with moisture and food materials. These experiments were carried out with material from numerous colonies and over a period of three years. Five hundred forty-five were used in groups of five to ninety. Of these 22.5 per cent underwent a moult and resorbed or lost their wing pads, thus coming to resemble large workers. Thirty-five individuals (6.7 per cent) became soldier nymphs or soldiers with wing pads reduced or missing. Eighty-two (15 per cent) became in appearance typical third-form reproductives, without wing pads and with a development of tan pigment over the body and particularly along the posterior margins of the thoracic plates. In numerous cases these individuals functioned in producing eggs—even though some colonies were short-lived. The remainder of the individuals died or remained unchanged. Experiments described later (Table 5) incidentally further confirm the soldier potentialities of the wing-padded types.

When isolated from the balanced population of the colony, then, these wing-padded forms undergo alterations which indicate that they possess both soldier and reproductive capacities and that they are not irreversibly determined by heredity to become exclusively reproductive nymphs of the second-form or of the first-form. In these experiments 44.2 per cent became either functional workers, soldiers, or third-form reproductives and may thus be said to have redifferentiated from their apparent alate tendencies. Of those which remained unchanged it is to be noted that most of them occurred in experiments which ran less than two months. It seems likely that a time-from-isolation factor is involved here but no accurate check was made on time-lapse between collecting and use of the material. In general, wing-padded individuals were used soon after being found.

These observations also indicate that in *Prorhinotermes simplex* (Hagen) the differentiation of nymphs into fixed sterile (soldier) and reproductive lines of development may occur at an extremely late date, barely before alateness sets in, and would seem to confirm suspicions based on observations of the body measurements previously described.

C. EFFECTS OF SOLDIERS ON DIFFERENTIATION OF NYMPHS

Various observers have noted, in the course of collecting in the field, that a tendency seems to exist towards maintenance of a constant pro-

portion of soldiers within termite colonies. Kalshoven states that in *Kalotermea tectonæ* the ratio is about one soldier to ten nymphs; in *K. flavicollis* the ratio was said by Grassi to be about 1:5 or 1:6; Harvey noted a range in *K. minor* of 1:12 to 1:25. Study of the data of all these authors indicates that there were relatively more soldiers present in small young colonies than in large, but the variations seem to be consistent. There is, of course, the possibility that in the mechanics of collecting, more chance for overlooking some soldiers existed in connection with the

TABLE 3
CASTE PROPORTIONS CENSUS
(From Large Colony Portions Stored in Laboratory)

THIRD-FORM REPRODUCTIVES	SOLDIERS	NYMPHS OR "WORKERS"	RATIO SOLDIERS: "WORKERS"	APPROX. TIME IN LABORATORY
47	210	781	1:3.7 ⁺	6 wks. or more
43	59	303	1:5.1	unknown
36	46	425	1:9.2	2 mo.
3	3	13	1:4.3	3½ mo.
4	60	109	1:1.8	9 mo. (originally started 1:2)
7	22	110	1:5.0	4 mo.
7	93	263	1:2.8	5 mo.
4	35	138	1:3.9	11 mo.
40	457	1280	1:2.5 ⁺	12 mo.
			1:4.2	Average Ratio

Standard Deviation = 2.036; Standard Error of Mean = .678; Standard Error of Deviation = .49⁺.

larger colonies, but these authors were not doing random collecting; special attention was being given to colony ontogeny, and some colonies were raised in the laboratories. These observations raise questions as to the mechanisms involved in maintaining such equilibria and focus attention again on Castle's hypothesis that soldiers in a colony exert a regulatory inhibiting effect on nymphs.

The soldier-worker ratio for *Prorhinotermes simplex* had not heretofore been investigated and the nature of the habitat in which this species is usually found makes it impractical to collect entire colonies for census purposes. But colony portions stored in the laboratory for considerable

time showed a tendency to approach a certain level of soldier population ratio. A summary of such colonies is recorded in Table 3. These counts are not, of course, as satisfactory as those on complete colonies would be, if obtainable, but in most of these instances the populations were thriving and reproducing, and appeared to be in a normal state of integration, so it is believed that such records constitute a fairly close approximation to the soldier balance characteristic for the species.

Furthermore, examination of the records kept for two months on forty-eight small groups of *Prorehinotermes* started without soldiers and ranging in size from five to seventy-five individuals, shows a trend towards a similar constant ratio. At the end of these experiments an average ratio of $1:3.5^+$ was reached. (Standard Deviation = 2.3; Standard Error = .55)

It is of interest to note also that in the preceding section dealing with the differentiation of wing-padded forms, soldiers appeared in the proportions of $1:3.6^+$ as compared with "worker-like" forms. Whether one is justified in lumping all small groups and using an average in this sort of study may, of course, be open to question, since there was no integrative continuity among the sample groups.

If we assume a tendency to maintain this fairly constant ratio, $1:3.5 \pm 0.55$, a very practical difficulty is reached in taking readings to check on this assumption: namely, how long does it take to reach the typical equilibrium in laboratory colonies started with too many or too few soldiers? No answer in terms of time is forthcoming at present; it is felt, however that the size of group and its success in adapting to the artificial laboratory conditions may be more important than a simple lapse-of-time factor. The factors of short duration of experiment and poor population growth are in these cases inseparably associated. Analysis of the data does show, however, that in 27 groups running one month the average ratio attained was $1:26$, whereas in 14 groups established twelve months or more the mean ratio of $1:4^+$ was reached. (The groups were started without soldiers.) Between these extremes no point can yet be indicated as a general time of reaching soldier-worker equilibrium.

This general analysis was made as a basis on which to construct experiments designed to test the inhibiting influence, if any, of soldiers on undifferentiated nymphs. It was obvious that if each soldier were to exert a certain inhibiting influence, such an influence would not be unlimited, but might be effective in a group of, for example, twenty-five and not in a larger group of one hundred. If one were arbitrarily to set up groups of one soldier to one hundred nymphs and compare them with groups of one hundred containing no soldier, and if one then found that soldiers

still developed in all groups it would not necessarily mean that soldiers exert no inhibiting influence; the influence might be present but undetectable due to the design of the experiment. After obtaining some indication of a probable normal soldier-worker ratio, it was believed logical to proceed in comparing groups lacking soldiers to those having soldiers in the ratio of 1:3 or 4. If any significant differences should now appear between such groups they can more safely be attributed to Castle's postulated soldier-inhibitor mechanism, knowing that in the control groups soldiers were present in approximately natural proportions.

In view of the possibilities discovered in regard to the nature of wing-padded individuals, and because of the relative accessibility of this instar, experiments were set up to investigate the developmental reaction of these forms to the presence of mature soldiers within their midst. Small groups of wing-padded nymphs were segregated into three-fourths-ounce jars and provided with moisture and wood, and with frass from their own colonies. Eight hundred and eighty individuals were segregated with soldiers in an appropriate ratio of one soldier to three nymphs; for comparison, eight hundred and seven individuals were set up simultaneously without soldiers present. The size of the groups ranged from five to ninety, as shown in Table 4, and some results of these experiments are therein summarized.

It may be noted that from a total of eight hundred and seven nymphs not associated with soldiers, fifty-seven (48.5 per cent) of those changing from the wing-padded condition developed into soldiers, whereas among eight hundred and eighty in contact with soldiers only six additional (2.5 per cent) occurred. When the figures are tested for significance of difference between proportions it is found that the actual difference (46.0 per cent) is over five times the standard error of the difference (2.75). Or, if the results be scrutinized in another way—by comparing the number of groups which developed soldiers in each category rather than by obtaining the total number of soldiers from experimental series—then it is found that there is an actual difference of 50.0 per cent in favor of the groups started without soldiers, and that this figure is over three and one-half times the standard error of difference (13.3).

This method of analysis, however, is open to criticism from the statistical viewpoint since the differences between the experimental and control series may be prejudiced by one or two favorable or unfavorable cases. Other approaches in analyses were therefore made.

Tables 5 and 6 present the same data in more concisely summarized fashion. Here emphasis is laid on the numbers and percentages of wing-padded individuals which changed to some other form; such cases are

for convenience termed "changelings." It is judged that only the changelings can give information in regard to differentiation path so the dead

TABLE 4
DIFFERENTIATION OF WING-PADDED FORMS
Part I. Groups Started Without Soldiers

ORIGINAL NUMBER	NUMBER DEAD	NUMBER NOT CHANGED	NUMBER BECOMING 3RD FORM REPROD.	NUMBER BECOMING SOLDIERS	NUMBER BECOMING WORKER- LIKE	TIME LAPSE
90	33	5	7	14	31	1 ½ mo.
63	21	17	2	12	11	1 ½ mo.
60	4	42	7	3	4	½ mo.
60	4	34	16	2	4	24 days
60	25	9	13	0	13	2 mo.
40	8	16	7	1	8	1 ½ mo.
30	6	18	4	1	1	1 mo.
30	8	18	2	1	1	1 mo.
30	4	13	10	1	2	1 mo.
30	7	16	3	1	3	1 mo.
30	14	6	7	0	3	1 ½ mo.
20	4	7	6	0	3	1 ½ mo.
20	8	1	1	0	0	1 ½ mo.
10	3	4	0	0	3	22 days
74+9 3rd	34	9	0	0	31	1 ½ mo.
30+6 3rd	11	5	0	2	12	1 ½ mo.
30+6 3rd	3	8	0	4	15	1 ½ mo.
30+6 3rd	10	10	3	5	2	1 ½ mo.
30+5 3rd	11	8	0	4	7	1 ½ mo.
20+4 3rd	5	5	0	5	5	2 mo.
20+3 3rd	3	12	1	1	3	1 ½ mo.
807	226	273	89	57	162	Totals
Percentages	28.0	30.3	11.02	7.06	20.07	

Total Per Cent Changed = 38.1

Per Cent of Changed which Became Soldiers = 18.5

and the unchanged should be excluded from calculations intended to test the general hypothesis that there is *no* influence of soldiers upon the further development of wing-padded nymphs.

In Table 6 it may be noted that considerable difference exists in the

TABLE 4—*Continued*

DIFFERENTIATION OF WING-PADDED FORMS

Part II. Groups Started With Soldiers

ORIGINAL NUMBER	NUMBER DEAD	NUMBER NOT CHANGED	NUMBER BECOMING 3RD FORM REPROD.	NUMBER BECOMING SOLDIERS	NUMBER BECOMING WORKER- LIKE	TIME LAPSE
63+17s.	28	3	10	0	22	1½ mo.
60+20s.	21	31	8	0	0	24 days
60+20s.	25	6	8	0	21	1½ mo.
60+20s.	2	50	5	1	2	½ mo.
31+10s.	11	7	3	0	10	1½ mo.
30+10s.	9	8	7	0	6	1½ mo.
30+10s.	0	24	5	1	0	½ mo.
30+10s.	8	16	5	0	1	1½ mo.
30+10s.	16	13	1	0	0	1½ mo.
30+10s.	0	29	0	1	0	10 days
30+10s.	6	20	2	1	1	1 mo.
30+10s.	7	17	4	1	1	1 mo.
30+10s.	4	23	2	0	1	1 mo.
30+10s.	20	7	3	0	0	2 mo.
30+10s.	18	8	4	0	0	1 mo.
30+10s.	10	5	5	0	10	1½ mo.
30+10s.	6	20	2	0	2	1½ mo.
30+10s.	13	6	7	0	4	1½ mo.
30+10s.	4	20	4	1	1	½ mo.
30+3s.	14	2	7	0	7	1½ mo.
20+10s.	1	18	1	0	0	10 days
20+10s.	5	11	3	0	1	1½ mo.
20+10s.	5	12	2	0	1	1½ mo.
20+5s.	3	11	4	0	2	1½ mo.
20+5s.	6	8	2	0	4	2 mo.
11+5s.	3	5	0	0	3	22 days
5+3s.	2	0	2	0	1	22 days
40+10s.+4 3rd	10	6	2	0	22	1½ mo.
880+288	257	386	108	6	123	Totals
Percentages	28.1	43.8	12.2	0.68	13.9	

Total Per Cent Changed = 26.9

Per Cent of Changed which Became Soldiers = 2.5

Actual difference in soldier percentages from Part I - Part II = 16.0

Standard error of difference = 2.75

number of changelings which became soldiers from groups of wing-padded only, as compared to the number becoming soldiers from the groups which already had soldiers present. The standard error of difference here is .029—which is about one-fifth the actual difference—and would seem to indicate significance in the data. However, this manner of lumping together

TABLE 5

DIFFERENTIATION OF WING-PADDED FORMS
(Analyzed as Individuals, Not Grouped)

Composition of Exp. Colonies	Total Orig. Number	No. Dead	No. Not Changed	Number of Changelings				No. of Groups Involved
				To 3rd Form	To Soldier	To Worker	Total	
Wing-padded only	573	149	216	85	36	87	208	14
Wing-padded + 3rd Forms	234 + 39	77	57	4	21	75	100	7
Wing-padded + Soldiers	840 + 278	247	380	106	6	101	213	27
Wing-padded + Soldiers + 3rd	40 + 10 + 4	10	6	2	0	22	24	1

TABLE 6

DIFFERENTIATION OF WING-PADDED FORMS
(Proportions of Individuals, Expressed in Fractions)

Composition of Exp. Colonies	No. Dead	No. Not Changed	No. Changed	Number Changelings Becoming:		
				3rd Form	Soldier	Worker
Wing-padded only	.2600	.3770	.3630	.4087	.1731	.4183
Wing-padded + 3rd Forms	.3291	.2436	.4273	.0400	.2100	.7500
Wing-padded + Soldiers	.2940	.4524	.2536	.4977	.0282	.4742
Wing-padded + Soldiers + 3rd	.2500	.1500	.6000	.0833	.0000	.8167

all individuals in the experiments may be questioned since it throws improper emphasis on the individual, when there is evidence throughout the work that in these social insects the groups may be reacting as a unit, with integrated functions amongst the individuals within the group.

Table 7, therefore, lays emphasis on the numbers of *groups* which produced soldier changelings and on the original group composition. In the

TABLE 7
DIFFERENTIATION OF WING-PADDED FORMS
(Analysis Using Original Groups as Units)

Final No. Changelings in Groups	NUMBER AND KINDS OF GROUPS HAVING CHANGELINGS					
	Originally with Wing-pads only (Situation No. 1)		Wing-padded + 3rd Form (Situation No. 2)		Wing-padded + Soldiers (Situation No. 3)	
	Not Prod. Soldiers	Producing Soldiers	Not Prod. Soldiers	Producing Soldiers	Not Prod. Soldiers	Producing Soldiers
1	1	0	0	0	2	1
2	0	0	0	0	0	0
3	1	0	0	0	5	0
4	0	1	0	0	3	1
5	0	0	0	1	0	0
6	0	1	0	0	3	3
7 or more	3	7	1	5	8	1

Chi-square values (Yates correction) from comparison of data situation No. 1 with No. 3:

Groups with 4 changelings = 5.21; Probability = 0.022

Groups with 6 changelings = 3.72; Probability = 0.053

Groups with 7 or more = 4.53; Probability = 0.033

first block, for example, the figure presented means that one group, which was originally composed of wing-padded nymphs only, produced one changeling and that this changeling was not a soldier. In the last block in the table the figure means that there was one group composed originally of wing-padded plus soldiers, which produced seven or more changelings and some of these changelings were soldiers. It was deemed unwise to attempt to draw conclusions in statistical analysis from any group in which there were less than seven changelings. With less than seven there could not be a 1:1 opportunity for the non-occurrence of soldiers, if we

assume soldiers appearing one-tenth of the time by chance alone.

Using the chi-square test on these data in Table 7, and applying Yates' correction because of the small numbers involved, it is found that when the 10 wing-padded groups having seven or more changelings are compared with the 9 wing-padded-plus-soldier groups from the same category, chi-square equals 4.53, and the probability that the results are due to

TABLE 8

INFLUENCE OF THIRD-FORM REPRODUCTIVES ON DIFFERENTIATION
OF WING-PADDED INDIVIDUALS
(Paired Experiments)

No. of Third-Form Reproductives Produced in Groups Started:		Differ- ences	No. of Soldiers Produced in Groups Started:		Differ- ences	Time Lapse in Days	Original Number Wing- Padded
Without Reprod.	With Reprod.		Without Reprod.	With Reprod.			
2	0	2	1	0	-1	60	10
3	1	2	0	1	1	42	20
4	0	4	1	5	4	60	20
3	0	3	1	4	3	51	30
7	0	7	0	2	2	51	30
4	3	1	1	5	4	52	30
4	0	4	1	2	1	50	30
2	0	2	1	0	-1	30	30
Mean Difference = 3.1			Mean Difference = 1.6				
P = .014 (Student's Method)			P = .017				

chance is about .033. Hence, the hypothesis that soldiers exert *no* influence appears doubtful in these experiments.

If the cases in "situation No. 2" (wing-padded plus third-form reproductives) be added to those in "situation No. 1" and the totals compared as before, it is found that the probability of getting the present results by chance alone is only .0048.

When groups in situation No. 1 are compared with those in No. 2 no evidence is found for the hypothesis that third-form reproductives stimulate the production of soldiers ($P = .65$). It is felt, however, that this

possibility cannot be completely rejected. Other analyses, summarized in Table 8, bring out the suggestion that while the presence of third-form reproductives did not significantly increase the *number of cases* (groups) in which soldiers differentiated, there was a significant increase in the *total number of soldier individuals* in colonies started with reproductives—as compared with controls originally lacking reproductives.

One other point is of interest: is there any effect of the presence of functional reproductives on the differentiation of additional reproductives from the wing-padded types? In Table 6 it may be observed that in colonies started with reproductives the proportion of changelings that became third-forms is strikingly different than in colonies of other types. This difference (.3687) has a standard error of .055 and is significant. Likewise, when the experiments are paired and examined by Student's method a probability of .014 is obtained and the hypothesis that functional reproductives may inhibit differentiation of nymphs in that direction seems applicable. Use of chi-square test on groups as units was not made here because calculated values fell below 5 in three cases (6.4, 0; .64, 2.1). Actual differences in terms of groups are readily apparent in the tabulation following:

STARTING CONDITIONS	NO. GROUPS PRODUCING REPRODUCTIVES	NO. GROUPS NOT PRODUCING REPRODUCTIVES
Wing-padded only	10	0
Wing-padded + 3rd-forms	1	6

Obviously, the rather small numbers presented in this phase of the studies make it urgent to repeat and extend the work, but trends and possibilities of considerable interest are at least suggested.

DISCUSSION

This investigation would seem to point again to the importance of dealing with living material over long periods of time when attempting analysis of caste differentiation problems. Had attention here been confined, as in Hare's work, to compilation of measurements of preserved forms taken at random from colonies, erroneous deductions would have followed in respect to the differentiation potentialities of wing-padded nymphs. It also seems beneficial to approach such a problem with the

idea of the supraorganism in mind in order to keep sufficiently aware of the importance of integration in relation to development of colony units. (Emerson, 1939; Wheeler, 1911.) On this basis the method here used involves fragmenting the colony (in a fashion analogous to that used by Child and others on single organisms) and then making observations on the reconstitution and reintegration of the units within the fragment. In *Prorehinotermes simplex*, isolated pieces of a colony are seen to "reconstitute" into small but balanced communities by the differentiation of some of the units into reproductives, soldiers, or workers. It is postulated that this reconstitution proceeds in such a fashion as to lead eventually to a balanced division of labor among workers, reproductives, and soldiers.

The observations being reported, although grouped into two phases, lead one eventually to recognize their interdependence. One should not generalize on the absolute size-range or number of instars in *Prorehinotermes* without specifying colony or environmental conditions. In one environment a nymph may, apparently, pass through six or seven instars and become an alate, but under other conditions a similar nymph may develop precociously into a wingless reproductive or a soldier. For instance, in experimental colony number 79-6, third-form reproductives and small, pigmented soldiers developed within one and one-half months from worker-like nymphs of twelve or thirteen antennal segments. This transformation involved at least one moult, but little increase in size. Thus the average measurements recorded in Figures 1 through 4 are simply representative of the various stages to be found in large natural colonies, and are not indicative of any fixed, undivertible progression in development. The existence of variations in head-widths and number of moults before differentiation in relation to colony age and size has already been described by Heath and Kalshoven for members of the Kalotermitidæ.

The present experiments, although contributing new knowledge concerning the caste problem in *Prorehinotermes*, do not delineate a complete picture; numerous questions are unanswered. For example, is there really a worker caste in *Prorehinotermes simplex*, as assumed by Thompson and Snyder (1920) and by collectors in general? Experiments with living forms show an unexpected plasticity among the supposed workers. There is, of course, the possibility that a distinct worker caste is present which is physiologically but not morphologically differentiated; my experiments do not settle that point.

Another question for future study centers around the possible influence of time or season on the plasticity of wing-padded forms. Alates have been collected only from October through January, but wing-padded

individuals—presumably the precursors of alates—are found from May through February. Now one wonders whether there may be a loss of plasticity in a given group of winged-padded forms with the passage of time toward the swarming season. Experience in handling specimens also now points to the importance of utilizing wing-padded forms soon after collection; after a storage period only the “less labile” individuals seem to remain, the others having changed into other forms.

With increasing evidence of the importance of extrinsic factors in directing differentiation there is increasing demand for description of the mechanisms involved. This demand is partially satisfied by Castle's (1934) and other's (Light, Hartman, and O. H. Emerson, MS) work showing the existence of an inhibiting, caste-specific, hormone-like substance acting through trophallaxis. But the part played by general nutritive conditions and interchanges within the colony, which have been little investigated, certainly should not be overlooked. Hungate (1938) analyzed the nutrition of *Zootermopsis* as individuals, but not in groups. The influence of size of groups on the rate and manner of reconstitution of the supraorganismic condition is not yet sufficiently understood. The cause of early appearance of soldiers and late development of alates during colony ontogeny (Heath, 1927; Kalshoven, 1930) needs further study; it seems probable that general nutrition as well as inhibition by hormone may be concerned. Perhaps also the reproductive inhibiting hormone suggested by Castle, or some other substance, may actually stimulate soldier development; the proximity of the founding queen to the first young in a new colony would then lead to the situation Heath records—in which all of the first year's individuals were said to belong to the soldier caste. The experimental evidence at hand supports such a suggestion. It may be observed from Table 8 that more soldiers appeared in those groups that were started with functional third-form reproductives, and that the differences between members of the pairs have a statistical significance of $P = .017$ when Student's method is used.

The experiments and observations herein described for *Prorhinotermes* are interpreted as favoring the extrinsic theory of caste differentiation. They also point to a situation which contrasts with that in certain other genera—at least in *Zootermopsis*, *Kaloterme*s, and *Reticulitermes*. In *Prorhinotermes simplex* the differentiation of nymphs into unalterable lines of reproductive and soldier (sterile) development, as judged by wing bud status, seems to occur at an extremely late date—just before the last instar. An attempt to emphasize these contrasts in diagram form is shown in Figure 5; these diagrams are based on information obtained to

date and are drawn from reports by Heath, Castle, Kalshoven, Grassi, and Hare. It may be assumed that nymphs hatch from the egg with reproductive, winged, and soldier potentialities. Undifferentiated growth is indicated by the broad, solid column. Points from which divergence may take place are indicated by the bases of the arrows and the final,

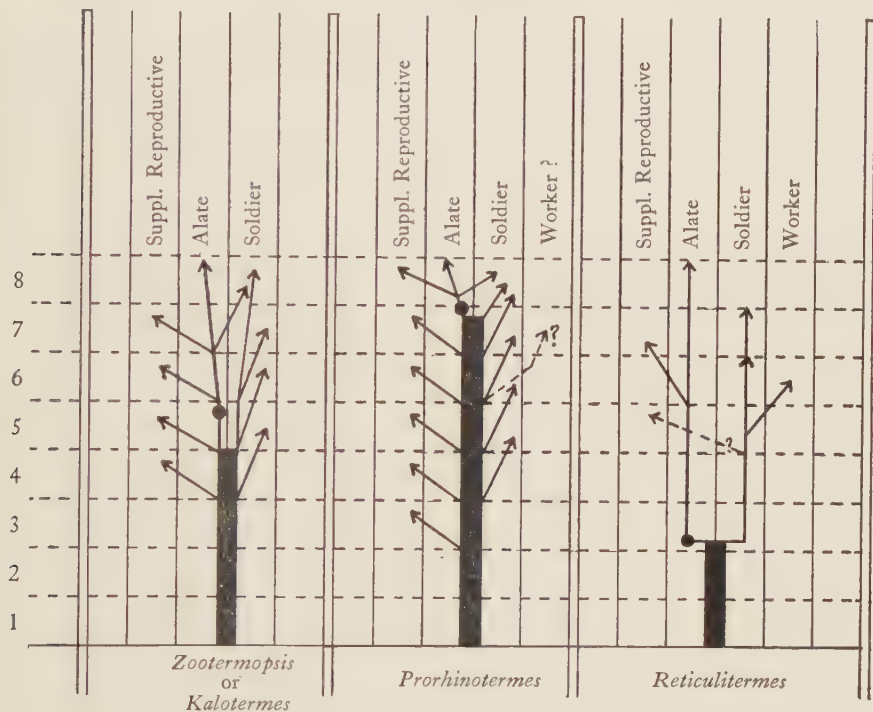


Fig. 5. Some possible lines of differentiation for nymphs in four genera of termites, as deduced from data in the literature and from evidence at hand. Numbers indicate stadia; solid column represents undifferentiated growth; arrows, ages at which differentiation may occur and paths which may be followed.

differentiated types by the positions of the heads of the arrows. The level or instar at which wing primordia are first externally distinguishable is marked for each genus with a black circle.

The relatively late differentiation and the prolonged "plasticity" of the nymphs might have been expected in the Kalotermitidae, in view of their other primitive properties, but it seems somewhat surprising to find *Prorhinotermes* — related morphologically to *Reticulitermes* — showing

such extreme modifiability at a late stage. One recalls, however, that in other instances social complexity and specialization of caste development do not always seem to run parallel to morphological specialization. The *Hodotermitidæ* provide a case in point.

SUMMARY

1. Head measurements were made on seven hundred and fifteen individuals representing all size classes of non-wing-padded nymphs from six colonies of *Prorehinotermes simplex* (Hagen). Frequency distribution curves constructed from these data indicate that there are six and possibly seven growth stadia. Other observations advance the likelihood that an eighth stage occurs before wings are formed. A constant antennal count is found to be associated with each distinct size group. The groupings are distinct from each other with the exception of that one whose individuals possess sixteen antennal segments; this group overlaps the fifteen and seventeen segment groups. Such a situation parallels that for the worker caste measurements as recorded by Hare in *Reticulitermes*, but experimental isolation of such individuals indicates their capacity to become supplementary reproductives. Hence, their status as a definitive worker caste is not clearly established. Mesothorax measurements on four hundred individuals led to similar conclusions.

2. No external morphological basis was discovered on which young nymphs can be recognized and separated as having only reproductive or only sterile potentialities. Observations on living colonies show that nymphs may become supplementary reproductives or soldiers even after wing pads are indicated.

3. The presence of adult soldiers in colonies of undifferentiated nymphs and of wing-padded forms had a slightly significant inhibiting influence on the development of additional soldiers. The influence appeared to be specific and was most clearly indicated when the ratio of soldiers to nymphs was approximately 1:5 or one to less than five.

4. In laboratory storage colonies and in collections from natural colonies a tendency towards a soldier-nymph equilibrium of 1:4 was indicated.

5. Under the conditions of these experiments, fragments of *Prorehinotermes simplex* colonies appear to be capable of reconstitution; this is accomplished by differentiation of nymphs of third instar or older or by redifferentiation of wing-padded individuals to provide the group with supplementary reproductives and soldiers.

6. The suggestion is made that in the eventual solution of the caste

differentiation problem the utilization of the isolation method for living material, though difficult, will prove valuable.

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